GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF CHLOROFORM EXTRACTS OF *JUSTICIA ADHATODA* LEAVES

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ABSTRACT

The presence of phytochemical constitutes has been reported from species of the Acanthaceae. The present study was designed to determine the bioactive compounds in the chloroform leaf extract of *Justicia adhatoda* using Gas Chromatography–Mass Spectrometry analysis. Gas chromatography-mass spectrometry (GC-MS) analysis of the chloroform leaf extract of *Justicia adhatoda* was performed by using Perkin Elmer Turbo Mass Spectrophotometer (GCMS-5975C, AGILENT, USA) outfitted with an auto sampler XLGC. The results revealed that, six possible compounds were identified using NIST library and their 2D and 3D structures were illustrated. Each compound has their own biological properties. They might have their individual medicinal and pharmacological efficacy.

KEYWORDS: *Justicia adhatoda*, Gas Chromatography, Mass Spectrometry, medicinal plants and bioactive.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant medicine can be complex [1].

Plants have been used as medicines from prehistoric times. The earliest records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines [2]. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, polyphenols, glycosides, and terpenes [3][4]. Medicinal plants are not only a major resource base for the traditional medicine & herbal industry but also provide livelihood and health security to a large segment of Indian population. The domestic trade of the AYUSH industry is of the order of Rs. 80 to 90 billion. The Indian medicinal plants and their products also account of exports in the range of Rs. 10 billion. There is global resurgence in traditional and alternative health care systems resulting in world herbal trade which stands at US$ 120 billion and is expected to reach US$ 7 trillion by 2050. Indian share in the world trade, at present, however, is quite low [5].

Fig. 1. ***Justicia adhatoda***

Justicia adhatoda, commonly known as Malabar nut, adulsa, adhatoda, vasa, or vasaka is shown in Fig 1. It is a medicinal plant native to Asia, widely used in Siddha Medicine, Ayurvedic, Homeopathy and Unani systems of medicine. It belongs to the family Acanthaceae [6]. *Justicia adhatoda* is a shrub with lance-shaped leaves 10 to 15 centimeters in length by four wide. They are oppositely arranged, smooth-edged, and borne on short petioles. When dry they are of a dull brownish-green colour [7]. They are bitter-tasting. When a leaf is cleared with chloral hydrate and examined microscopically the oval stomata can be seen. They are surrounded by two crescent-shaped cells at right angles to the ostiole. The epidermis bears simple one- to three-celled warty hairs, and small glandular hairs. Cystoliths occur beneath the epidermis of the underside of the blade. The trunk has many, long, opposite, ascending branches, where the bark is yellowish in colour. Flowers are usually white and the inflorescence shows large, dense, axillary spikes. Fruits are pubescent, and are with club-shaped capsules [8].

J. adhatoda is a small tree which flowers in cold season. There is a saying is Sanskrit about this drug meaning of which is that as long as Vasaka will remain, patients suffering from spitting of blood, phthisis and common cold and cough need not despair. The physicians confidently proclaim that no death can take place from cough of any kind if Vasaka can play its role and find time to display its healing properties. Traditionally it has long been used as a great remedy for respiratory disorders. *J. adhatoda* acts as expectorant and relieves congestion and dyspnoea. It is used to treat fever, jaundice, tuberculosis, and headache. It protects the body from radiation damage caused during cancer treatment. It is used to treat urinary infections, vaginal discharge, pregnancy pain and fever. It acts as a counterirritant for Inflammatory Swelling. It is also used as abortifacient [9].

Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analysed by GC/MS it must be sufficiently volatile and thermally stable [10].

MATERIALS AND METHODS

Collection of Plant Materials

Justicia adhatoda leaves were collected from Kumarcoil, Kanyakumari district during the month of January - February in the year 2017.

Preparation of Powder and Extract from Leaves

The leaves were separated and cleaned well. Cleaned leaves were then dried under shade was shown in Fig 2. The drying process was continued until all the water molecules evaporated and leaves became well dried for grinding. After drying, the leaves were ground well using mechanical blender into fine powder and transferred into air tight container with proper labelling for further use. The dried and powdered *Justicia adhatoda* leaves were extracted with Chloroform using Soxhlet apparatus.

Fig.2. *Justicia adhatoda* dried leaves

After incubation excess petroleum ether was decanted and kept for drying. The dried samples were wrapped in muslin cloth and were kept for soxhlet extraction in 300 ml of solvent at boiling point of increasing polarity. Solute thus separated were collected in an eppendorf tube and used for further studies

Characterization of *Justicia adhatoda* Chloroform leaf Extracts

Justicia adhatoda chloroform leaf extracts were subjected to Gas chromatography mass spectrometry (GC-MS). GC-MS examination was completed by using Perkin Elmer Turbo Mass Spectrophotometer (GCMS-5975C, AGILENT, USA) outfitted with an auto sampler XLGC were shown in Fig.3.4. The column utilized was Perkin Elmer Elite-5 capillary column (dimethyl polysiloxane, 30m × 0.25 mm) with a film thickness of 0.25 mm. The bearer gas utilized was Helium at a 1.5 ml/min flow rate. The sample infusion volume used was1µl. The injector temperature was maintained at 250° C. The oven temperature was altered, initially at 70° C for 3 minutes and afterward customized to increment to 300° C in steps of 10°C. The overall run time was 35 minutes. The MS exchange line was kept at a temperature of 240° C. It was recorded utilizing electron splash ionization at 70 eV and information was assessed utilizing all out particle number (TIC) for compound distinguishing proof and evaluation. The spectra of the parts were contrasted and otherworldly database of known segments in the GC-MS library (NIST- 11). Estimation of crest zones and information handling were done by Turbo-Mass-OCPTVS-Demo SPL programming.

RESULTS AND DISCUSSION

Characterization of *Justicia adhatoda* leaf Extracts

The chloroform leaf extract of *Justicia adhatoda* were subjected to GC-MS analysis. The GC-MS spectra of six possible compounds were shown in Fig. 3 and tabulated in Table 1.

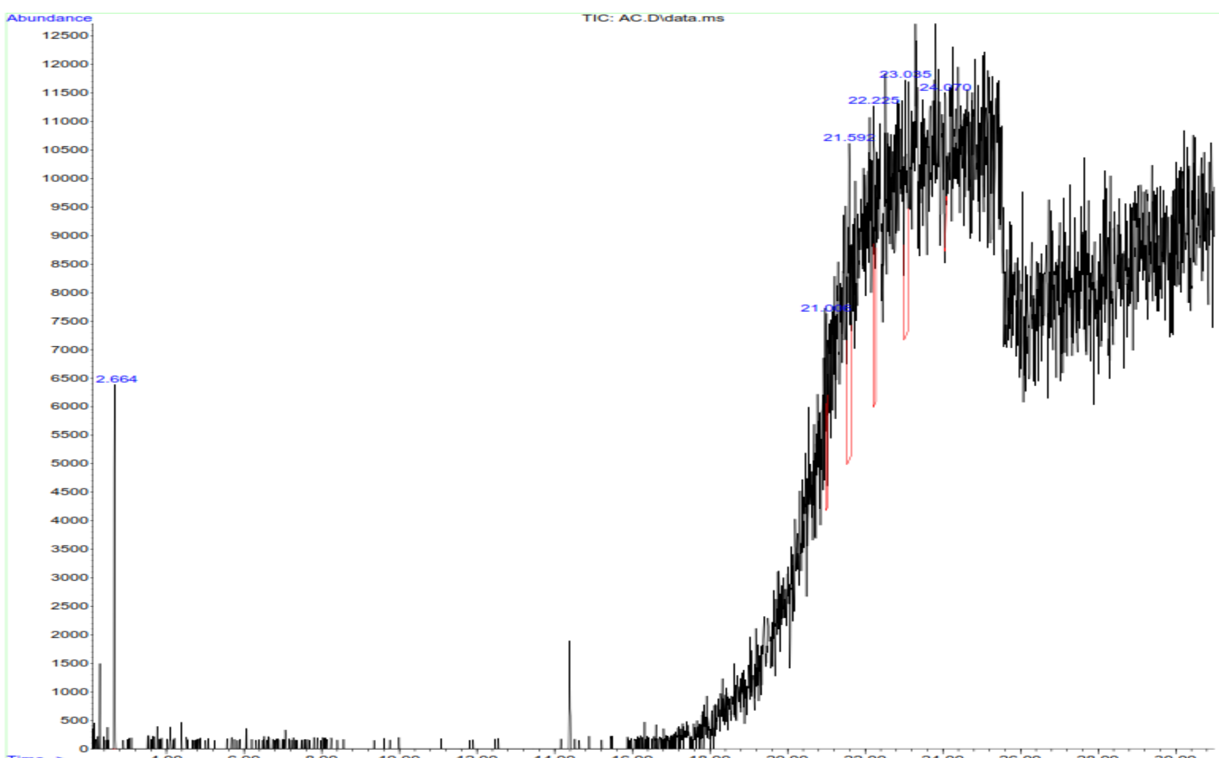


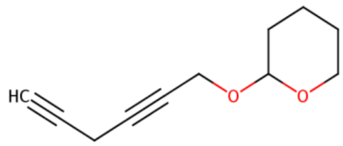
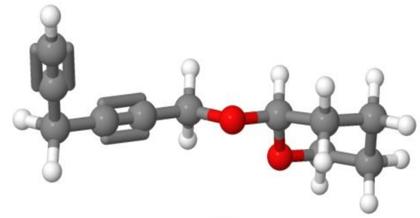
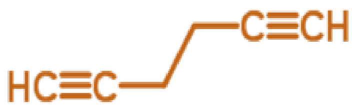
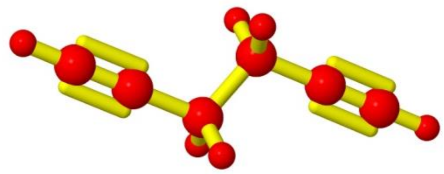
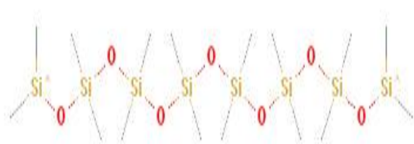
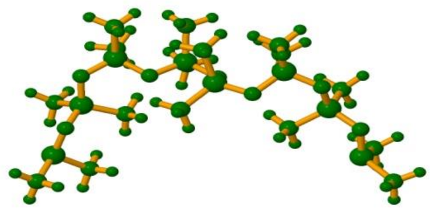
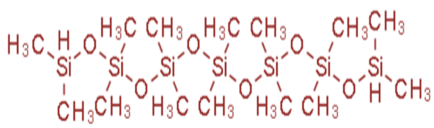
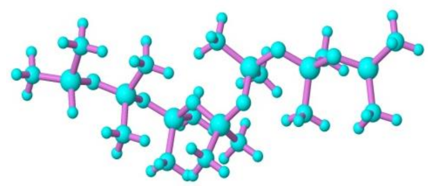
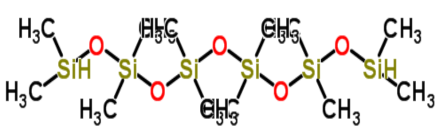
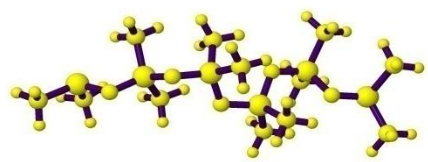
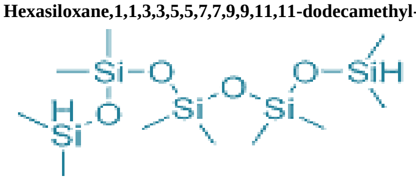
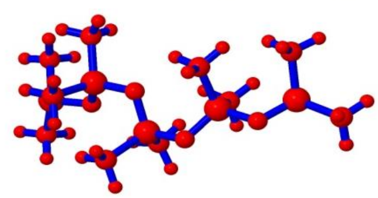
Fig. 4.5. GC-MS spectra showing six possible compounds

Table. 1. Six possible compounds from Justicia adhatoda chloroform leaf extracts

S.No	Compound Name	Molecular Formula	Molecular Weight (g/mol)
1	2-(2,5-Hexadiynyloxy)tetrahydro-2H-pyran	$C_{11}H_{14}O_2$	178.231
2	1,5-Hexadiyne	C_6H_6	78.114
3	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15-Hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577.233
4	Heptasiloxane,1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	505.09406
5	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	430.940
6	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-	$C_{10}H_{32}O_4Si_5$	357

The six possible compounds were identified using NIST library and their 2D and 3D structures were illustrated in Table 2. The main aim of the study is to characterize the bioactive chemical compounds from *Justicia adhatoda* chloroform leaf extracts. The six possible bioactive chemical compounds were identified and their 2-Dimentional (2D) and 3-Dimensional (3D) structures were also illustrated in the Table. 2. Each compound has their own biological properties. They may have their individual medicinal and pharmacological efficacy. 1,5-Hexadiyne have the lowest molecular weight as 78.114 whereas the Octasiloxane,1 , 1 , 3 , 3 , 5 , 5 , 7 , 7, 9 , 9 , 11 , 11 ,13 ,13 ,15 ,15 -Hexadecamethyl- have highest molecular weight as 577.233.

Table 4.7. 2D & 3D structures of six possible compounds from *Justicia adhatoda* chloroform leaf extracts

S.No	2D Structure	3D Structure
1	 2-(2,5-Hexadiynyloxy)tetrahydro-2H-pyran	
2	 1,5-Hexadiyne	
3	 Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-	
4	 Heptasiloxane,1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	
5	 Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	
6	 Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-	

Justicia adhatoda leaf extracts prepared in chloroform solvent were analyzed the phytochemical profile using Gas Chromatography - Mass Spectrometry method. GC-MS analysis of *J. adhatoda* leaf extracts revealed the existence of the major peaks presented in chloroform extracts were Amrinone (RT: 15.88); n- Hexadecanoic acid (RT: 16.33); Phytol (RT: 17.81); 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (RT: 18.04). From the study it is palpable that *J. adhatoda* leaf extracts contains many bioactive compounds and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drugs and Insecticides [11].

The traditional use of the plant *Justicia adhatoda* for the treatment of infectious diseases is promising, mainly against bacteria and fungi. Purification of the Bio-active components from the extracts understands the possible antimicrobial activities. GC-MS result signified the presence of six phytochemical components. The result of the study offers the platform of using *Justicia adhatoda* leaves as herbal alternatives for various diseases and it is highly significant for mosquitocidal activities. The result revealed in the current study partially correlates with the pre available GC-MS data. Both the results were partially matched to the number of compounds in the NIST library [12].

CONCLUSION

Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemicals which may vary depending on various factors, one amongst is the geographical localities which show quantitative variation in their chemical constituents. The study also provides a strong evidence for the use of *Justicia adhatoda* leaf extracts to treat for various diseases as a therapeutic agent [13]. The GC-MS study revealed that there are six possible potential phyto-components present in the chloroform leaf extract. The commotion may be due to the presence of one or more phytochemical constituents present in the extract. However, further studies have to be extended for other pharmacological studies [14].

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